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SYNTHETIC ACCESS TO POLY-SUBSTITUTED 11*H*-PYRIDO[3,2-*a*]CARBAZOLES, A DNA-INTERCALATING ELLIPTICINE RELATED STRUCTURE, AND THEIR ANTIPROLIFERATIVE ACTIVITY

Ming-Yu Wu,[1,5] Elkhabiry Shaban,[1] Marta Świtalska,[3] Ning Wang,[1] Miho Shimoda,[1] Yusuke Mizutani,[4] Megumi Yoshida,[1] Zhen-Wu Mei,[1] Hiroyuki Kawafuchi,[2] Junzo Nokami,[4] Joanna Wietrzyk,[3]* Xiao-Qi Yu,[5] and Tsutomu Inokuchi[1]*

[1]Division of Chemistry and Biotechnology, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan, [2]Toyama National College of Technology, Hongo-machi, Toyama, 939-8630, Japan, [3]Institute of Immunology and Experimental Therapy, Polish Academy of Science, 12, R. Weigl Street, 53-114 Wroclaw, Poland, [4]Department of Applied Chemistry, Faculty of Engineering, Okayama University of Science, Ridai-cho, Kita-ku, Okayama, 700-0005, Japan, [5]Key Laboratory of Green Chemistry & Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu 610064, P. R. China

E-mail*: inokuchi@cc.okayama-u.ac.jp, wietrzyk@iitd.pan.wroc.pl

Abstract − The facile procedure for the synthesis of the 11*H*-pyrido[3,2-*a*]carbazole structure involving the Fischer indole cyclization on tetrahydroquinolinones, available from enaminones and methyl 2-formyl-3-oxopropanoate, followed by the aromatization of the resulting 5,6-dihydro derivatives is described. This method allows for the introduction of substituents at C2, C6, and C8 to the scaffold by choice of the starting materials. In the biological testing, introduction of the phenyl group at C6 is significantly effective to improve the antiproliferative activity.

INTRODUCTION

The natural plant alkaloids with a linearly condensed aromatic ring system show interesting antitumor activities due to their DNA intercalating ability.[1] For example, ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, **I**, Figure 1), an alkaloid isolated from Apocyanaceae plants,[2,3] exhibits significant antitumor, anti-HIV,[4a] and anti-malarial activities.[4c] Accordingly, the overlapping interaction of the 6*H*-pyridocarbazole aromatic rings with that of a DNA base pair was precisely demonstrated.[5] Nevertheless, ellipticine **I** and its derivatives are mutagenic to the *Salmonella typhimurium* Ames tester strains, *Neurospora crassa*, and mammalian cells.[4a,4b,6]

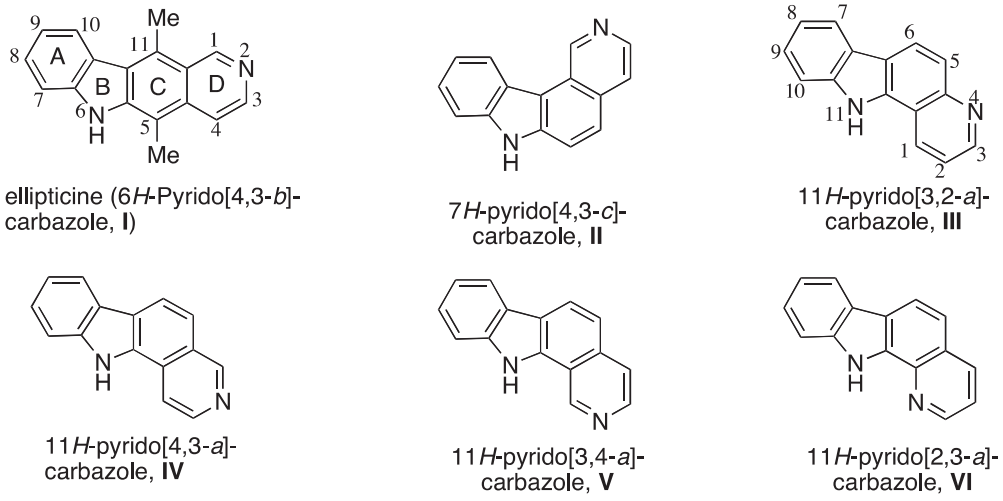


Figure 1. Structures of biologically relevant natural and synthetic pyridocarbazoles

The DNA intercalating and antitumor ability of the related structures, such as 11*H*-pyrido[3,2-*a*]-, [4,3-*a*]-, [3,4-*a*]-, and [2,3-*a*]-carbazoles **III**~**VI** were subsequently tested, indicating that the cytotoxicity of the 11*H*-pyridocarbazoles, measured on L1210 cells in vitro, is much lower than those of the 6*H*- and 7*H*-pyridocarbazole analogues **I**, **II**.[7] However, a comparative study of the interaction of the pyridocarbazole analogues with DNA topoisomerase II has not been well examined. Furthermore, the 3-substituted 11*H*-pyrido[3,2-*a*]carbazole derivatives were shown to possess slight antidepressant and L-dopa-potentiating effects.[8]

Syntheses of ellipticine and its related pyridocarbazole analogues were mainly relied on cyclization of hydrazinoquinoline with cyclohexanone via the Fischer-Borsche indole synthesis followed by aromatization or the Skraup reaction of aminocarbazoles with acrolein.[9,10] A Doebner reaction was applied to form 1-carboxy-3,5-dimethyl-7*H*-pyrido[2,3-*c*]carbazole from 3-amino-2-methylcarbazole,

acetaldehyde, and pyruvic acid.[11] The C ring of ellipticine core are constructd by the cyclization of *o*-quinodimethane intermediate, generated by thermolysis of the 2-alkyl-3-[α-(3-pyridyl)vinyl]indole.[12,13] Regioselective acylation of a 3-lithio-l-(phenylsulfonyl)indole with 3,4-pyridinedicarboxylic anhydride to the corresponding quinone was demonstrated.[14] A one-step procedure was developed by the Diels-Alder reaction between 1,4-dimethylpyrano[3,4-*b*]indol-3-one and 3,4-didehydropyridine to construct ellipticine core.[15]

For the synthesis of the 11*H*-pyridocarbazoles core, the Fischer indole cyclization of the 7-(azanaphthalenyl)hydrazines with 4-methoxycyclohexanone followed by aromatization,[16–19] or the reaction of 7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione with phenylhydrazine followed by aromatization,[8] or the electrocyclization of the 1-(3-indolyl-2-(pyridyl)propenes under UV irradiation have been reported.[20,21] However, these procedures suffer from low yields during the aromatization steps and are not feasible to supply the corresponding pyridocarbazoles with various substituents in the further structure–activity relationship (SAR) studies.

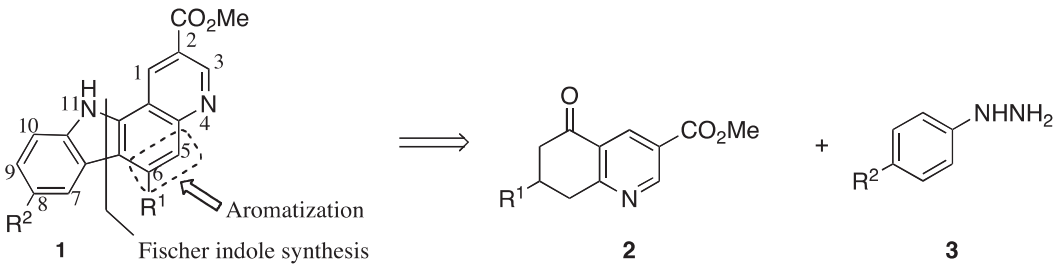


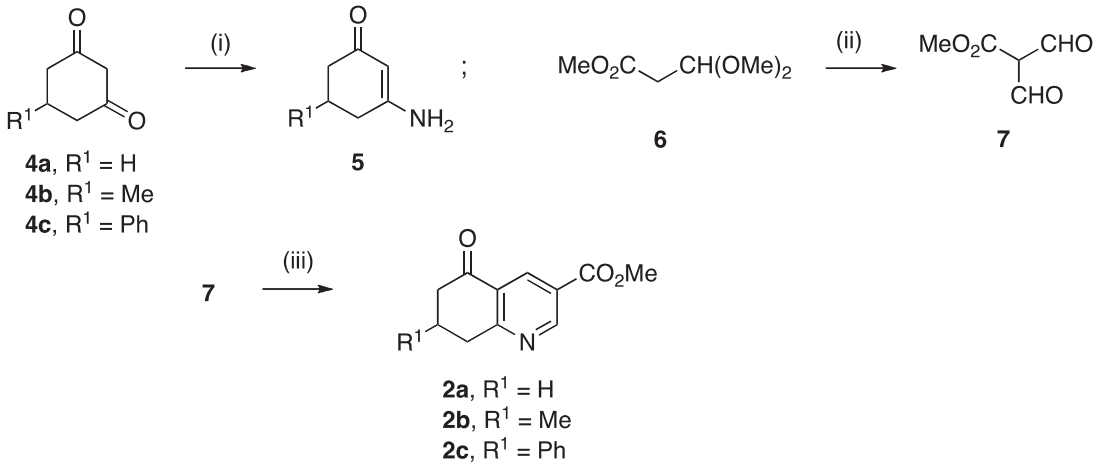
Figure 2. Synthetic plan to 2-methoxycarbonyl-11*H*-pyrido[3,2-*a*]carbazoles by Fischer indole synthesis followed by aromatization

In this study, we developed a synthetic access to the 2-methoxycarbonyl-11*H*-pyrido[3,2-*a*]carbazoles **1** by the Fischer indole synthesis of the corresponding 2-methoxycarbonyltetrahydroquinolinones **2** with phenylhydrazines **3**.[22–24] The method described here would permit formation of 11*H*-pyrido[3,2-*a*]carbazole scaffold with an ester group at the C2 and additional option of substituents at the C6 and C8. Availability of poly-substituents in the pyrido[3,2-*a*]carbazole core could be useful for further SAR study toward prospective anticancer and antimalarial activities (Figure 2). In addition, preliminary biological testing of the some obtained compounds was performed against MV4-11 cell line (human leukemia).

RESULTS AND DISCUSSION

Synthesis of tetrahydroquinolinone derivatives and their biological properties as group I mGluR antagonists are claimed in the patent by Jirgensons et al.[25] However, we prepared the starting 2-methoxycarbonyltetrahydroquinolinones **2** by condensation of the enaminones **5** with methyl 2-formyl-3-oxopropanoate (**7**), according to the method reported by us.[26]

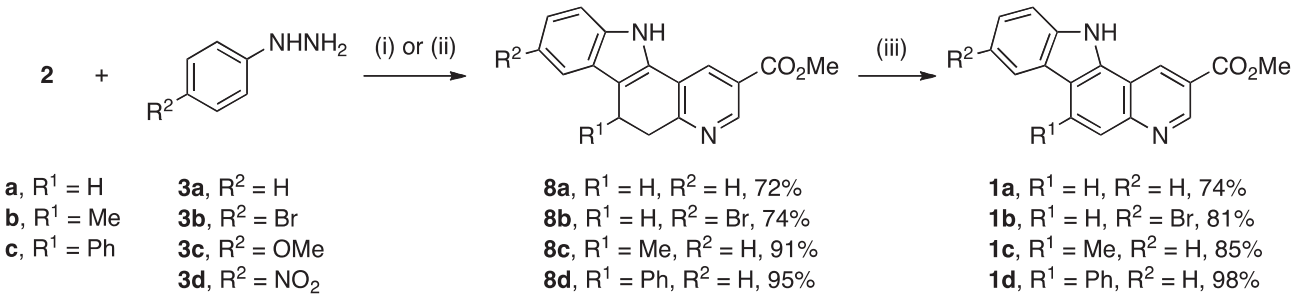
Thus, **5** were formed by the reaction of the 5-substituted cyclohexane-1,3-diones **4** with ammonium acetate while heating in toluene.[27,28] Their counterpart **7** was prepared by the Claisen condensation of methyl 3,3-dimethoxypropionate **6** with methyl formate using NaH in ether followed by hydrolysis with 10% HCl. The two-component [3+3] cycloannulation process for the rapid formation of the desired tetrahydroquinolinones **2** was achieved in an one-pot operation by the tosylation of **7** with TsCl-Et_3N forming the corresponding 2-formyl-3-(tosyloxy)acrylate, followed by the addition of **5** in the presence of pyridine, affording 2-methoxycarbonyltetrahydroquinolones **2** in moderate yields (Scheme 1).



Scheme 1. Synthesis of tetrahydroquinolinones **2**. Reagent and conditions: (i) $AcONH_4$, toluene; (ii) (a) HCO_2Me, NaH, Et_2O, (b) HCl; (iii) (a) Et_3N, then *p*TsCl in DMF, (b) **5**, pyridine, DMF

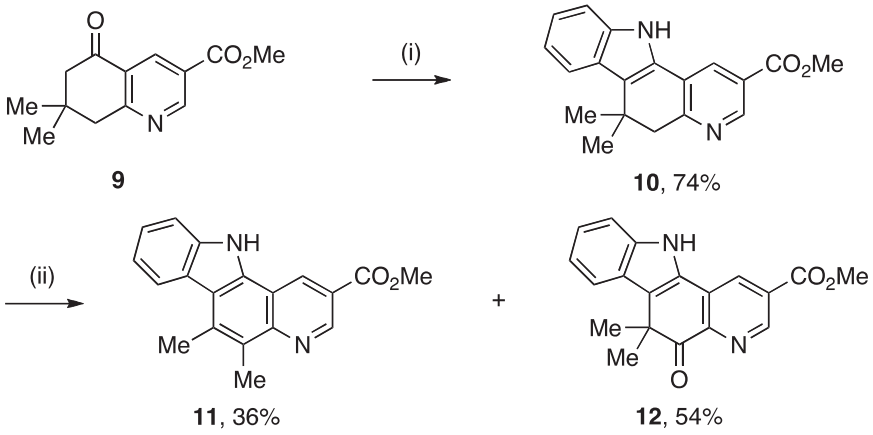
The Fischer indole cyclization of **2a** (R^1 = H) using phenylhydrazine **3a** (R^2 = H) was performed by heating in acetic acid with 36% HCl, affording the desired tetracyclic 6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazoles **8a** in 72% yield. A similar indole synthesis on **2a** (R^1 = H) was successfully achieved with 4-$BrC_6H_4NHNH_2$ **3b** (R^2 = Br), while the use of 4-$NO_2C_6H_4NHNH_2$ **3d** (R^2 = NO_2) resulted in no desired indole structure. The oxidative dehydrogenation of **8a** to the desired 11*H*-pyrido[3,2-*a*]carbazole **1a** was achieved by the treatment with DDQ in benzene in 74% yield. The

substituent R^1 affected the conversion of the DDQ oxidation; a high yield was attained with Ph group (**1d**, 98% yield), while a slightly lesser yield with Me group (**1c**, 85%) (Scheme 2).



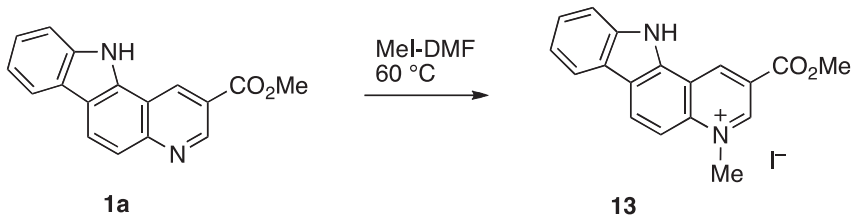
Scheme 2. Synthesis of substituted 11*H*-pyrido[3,2-*a*]carbazoles **1**. Reagent and conditions: (i) AcOH-36% HCl, 118 °C (for **8a** and **8b**); (ii) (a) AcOH-36% HCl, 118 °C, (b) MeOH-$SOCl_2$, 118 °C (for **8c** and **8d**); (iii) DDQ, benzene, reflux

We subsequently examined the preparation of 5,6-dimethyl-11*H*-pyrido[3,2-*a*]carbazole **11** through the 1,2-rearrangement of the *gem*-dimethyl group of the precursor **10** during the aromatization step.[29–31] Thus, the tetrahydroquinolinone **9**, derived from the dimedone in a manner described in the literature,[26] was converted to the corresponding 6,6-dimethyl-6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole **10** by the Fischer indole cyclization with hydrazine **3a**. The oxidation of **10** with SeO_2 (2 equiv.) in MeCN-H_2O afforded the desired **11** (36%) and the ketone **12** (54%) as a by-product. In all our attempts, the oxidation of **10** with DDQ, the reaction was sluggish and a hardly separable mixture of the starting **10** and the aromatized **11** was produced. The formation of **11** can be explained by the Wagner-Meerwein type rearrangement of the methyl group followed by aromatization during the oxidation process with SeO_2 (Scheme 3).



Scheme 3. Synthesis of 5,6-dimethyl-11*H*-pyrido[3,2-*a*]carbazole **11**. Reagent and conditions: (i) a) **3a**, AcOH-36% HCl, 118 °C, b) MeOH-$SOCl_2$, 118 °C; (ii) SeO_2, MeCN, reflux

In connection of search for finding biologically significant derivatives, the 11*H*-pyrido[3,2-*a*]carbazoles **1a** was converted its pyridinium salt **13** by treatment with MeI in DMF on heating, quantitatively (Scheme 4).



1a **13**

Scheme 4. Formation of pyridinium salt **13**

Antiproliferative activity in vitro against MV4-11 cell line

In order to study the biological properties of 11*H*-pyrido[3,2-*a*]carbazoles, we tested antiproliferative activity of prepared compounds against human leukemia MV4-11 cells and the results are summarized in Table 1. The results of the cytotoxic activity in vitro were expressed as IC_{50} (in μM), the concentration of the compound that inhibits proliferation of the cells by 50% as compared to the untreated control cells. Except for **1d** and **12**, other compounds show no-activity against MV4-11 cells. We modified structure of **1a** by introducing Br group at C8 and Me group at C6, but those groups are not effective. Introduction of the Ph group at C6 significantly improve the antiproliferative activity. The presence of two Me groups at C5 and C6 is also not effective. Some literatures reported that introduction Me group at N atom of the indole or quinoline ring can improve the biological activity of the compound,[32,34] but the compound **13**, pyridinium bearing the methyl group at the N4 of the quinoline ring, still showed no activity. However, 5-ketone compound **12** showed antiproliferative activity. According to SAR study, introduction of an amino group is a promising method to improve the biological activity,[32,34] so in the future, we will modify the 11*H*-pyrido[3,2-*a*]carbazole core with the amino group and varying other substituents at C8 or C6.

Table 1. Antiproliferative activity of neocryptolepine analogues against MV4-11 human leukemia cell line. IC_{50} value, μM

Compounds	IC_{50}, μM (μg/mL)
1a	NA[a]
1b	NA[a]
1c	NA[a]
1d	6.75±0.60 μM (2.38±0.21)
11	NA[a]
12	15.17±3.28 μM (4.86±1.05)
13	NA[a]

[a]NA- not active, IC_{50} above 10 μg/mL

In summary, we have described a quick access to poly-substituted 11*H*-pyrido[3,2-*a*]carbazoles **1** by the Fischer indole cyclization on 5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate followed by aromatization. Due to high activity of the saturated C5–C6 bond, flanked by two aromatic rings of the resulting 6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate **8**, the subsequent DDQ oxidation smoothly proceeded to afford the desired **1** in good yields. When this aromatization was applied to the 6,6-dimethyl derivative **10**, the 1,2-rearrangement of a methyl group was induced with SeO_2 to produce the corresponding 5,6-dimethylated 11*H*-pyrido[3,2-*a*]carbazole **11**. Thus, we developed a new procedure to introduce an alkyl group to the C5 position. Introduction of the phenyl group at C6 is significantly effective to improve the antiproliferative activity. Further study of the derivatives available from the 11*H*-pyrido[3,2-*a*]carbazole motif in order to improve their anticancer activities is currently underway in our laboratory.

EXPERIMENTAL

General Procedures

The commercially obtained reagents were directly used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254) and flash chromatography was performed on silica gel (230–40 mesh) using a gradient solvent system. (hexane/ ethyl acetate as the

eluent unless otherwise specified). The ^{1}H NMR and ^{13}C NMR spectra were measured on the Varian INOVA-400 spectrometer. Melting points were determined on a J-Science RFS-10 hot stage microscope. High resolution mass spectra were obtained on a Bruker micrOTOF II-SKA spectrometer.

Preparation of methyl 6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (8a), a general procedure (Method A for 8a and 8b): A mixture of **2a** (205 mg, 1.0 mmol) and phenylhydrazine (**3a**, 130 mg, 1.2 mmol) in AcOH (4 mL)-36% HCl (1 mL) was heated under reflux for 5 h. After the reaction completed, a small amount of ice water was added, then the mixture was neutralized with saturated aqueous $NaHCO_3$. Products were extracted with EtOAc (30 mL x 3), and the extracts were washed with brine, dried over Na_2SO_4, and concentrated on an evaporator. The crude product was used for the next step without further purification.

Fischer indole cyclization and the subsequent esterification, giving 8c, 8d, and 10 (Method B): A mixture of **2b** (1.0 mmol) and **3a** (1.2 mmol) were dissolved in AcOH-HCl (4:1, 5 mL) and heated for 5 h under reflux. After the reaction completed, the yellow solids were collected by filtration. The solids thus obtained were dissolved in MeOH (10 mL) and $SOCl_2$ (238 mg, 2 mmol) was added. The mixture was stirred under reflux for 20 h. The solvents were evaporated under reduced pressure, a small amount of water was added, and neutralize to pH 8 with 1N NaOH. The solids **7c** obtained by filtration was dried in vacuum overnight.

Methyl 6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (8a): Pale yellowish solids in 72% yield, mp 239–241 °C (after LC); IR (KBr) ν_{max} = 3225, 2947, 2889, 2839, 1937, 1898, 1821, 1728, 1601, 1551, 1481, 1441, 1440, 1362, 1317, 1294, 1244, 1119, 1051, 1009, 993, 978, 922, 910, 870, 810, 760, 748 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.39 (s, 1H), 8.16 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.25 (t, *J* = 7.2 Hz, 1H), 7.16 (t, *J* = 7.2 Hz, 1H,), 3.98 (s, 3H), 3.34 (t, *J* = 8.0 Hz, 2H), 3.15(t, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 161.9, 147.1, 138.3, 130.9, 127.6, 126.6, 125.3, 124.5, 123.0, 119.8, 119.4, 112.5, 112.1, 52.8, 32.4, 18.9. HRMS (ESI) calcd for $C_{17}H_{15}N_2O_2$ $(M+H)^+$: 279.1134, found: 279.1128.

Methyl 8-bromo-6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (8b): Pale yellowish solids in 74% yield, mp 293–295 °C (after LC); IR (KBr) ν_{max} = 3229, 2953, 2841, 1886, 1822, 1726, 1616, 1601, 1550, 1487, 1443, 1397, 1360, 1312, 1296, 1248, 1220, 1128, 1041, 1013, 990, 972, 934, 968, 812, 764

cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 8.79 (s, 1H), 8.48 (s, 1H), 7.72 (s, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 3.91 (s, 3H), 3.21 (t, *J* = 8.0 Hz, 2H), 3.03 (t, *J* = 8.0, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 161.6, 147.1, 136.5, 131.9, 128.0, 127.6, 124.9, 124.3, 124.0, 121.2, 113.5, 111.8, 111.6, 52.3, 31.7, 18.2. HRMS calcd for $C_{17}H_{13}BrN_2O_2$: 356.0160, found: 356.0201.

Methyl 6-methyl-6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (8c): yellow solids in 91% yield of the two steps, mp 163–165 °C (after LC); IR (KBr) ν_{max} = 3555, 3142, 2953, 2482, 2012, 1892, 1730, 1713, 1605, 1553, 1479, 1441, 1412, 1364, 1323, 1308, 1290, 1277, 1233, 1136, 1076, 1049, 117, 980, 922, 882, 812, 764, 743 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 8.81 (d, *J* = 1.6 Hz, 1H), 8.56 (d, *J* = 1.6 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 3.92 (s, 3H), 3.46–3.35 (m, 2H), 3.03 (dd, *J* = 16.4, 7.2 Hz, 1H), 1.24 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.7, 160.7, 146.4, 138.5, 129.6, 128.1, 126.0, 125.1, 124.9, 123.1, 119.9, 119.7, 118.0, 112.2, 52.9, 26.2, 21.4. HRMS calcd for $C_{18}H_{16}N_2O_2$: 292.1212, found: 292.1182.

Methyl 6-phenyl-6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (8d): yellow solids in 95% yield of the two steps, mp 233–235 °C (after LC); IR (KBr) ν_{max} = 3096, 2499, 2002, 1890, 174, 1630, 1564, 1497, 1454, 1439, 1417, 1352, 1325, 1292, 1215, 1126, 109, 985920, 895, 814, 743 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 8.77 (d, *J* = 13.6 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.24–7.19 (m, 5H), 7.13 (t, *J* = 8.0 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.88 (t, *J* = 7.6 Hz, 1H), 4.70 (t, *J* = 7.2 Hz, 1H), 3.95 (s, 3H), 3.71 (dd, *J* = 16.8, 7.6 Hz, 1H), 3.45 (dd, *J* = 16.8, 6.4 Hz, 1H). HRMS calcd for $C_{23}H_{18}N_2O_2$: 354.1368, found: 354.1369.

Methyl 6,6-dimethyl-6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (10): Pale yellowish solids in 74% yield, mp 217–220 °C (after LC); IR (KBr) ν_{max} = 3433, 3096, 2949, 2498, 2021, 1904, 1744, 1630, 1560, 1499, 1456, 1416, 1325, 1304, 1267, 1223, 1194, 1136, 1119, 1096, 1034, 1013, 980, 924, 882, 814, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.86 (s, 1H), 8.83 (t, *J* = 2.0 Hz, 1H), 8.61 (d, *J* = 2.0 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 3.94 (s, 3H), 3.13 (s, 2H), 1.43 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.4, 158.4, 142.9, 138.4, 129.7, 127.9, 125.7, 125.6, 124.7, 123.0, 121.5, 120.5, 119.6, 112.0, 52.7, 45.9, 32.9, 28.4(2C). HRMS calcd for $C_{19}H_{18}N_2O_2$: 306.1368, found: 306.1319.

Preparation of methyl 11*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (1a), a general procedure: A

mixture of **8a** (140 mg, 0.5 mmol) and DDQ (227 mg, 1.0 mmol) was dissolved in benzene (10 mL) and then heated at reflux for 4 h under the monitoring of TLC (hexane:THP = 2:1). After the reactions completed, the reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL) and extracted with EtOAc (30 mL x 3). The organic phase was collected and washed with saturated aqueous $NaHCO_3$ and brine to give **1a**.

Methyl 11*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (1a): Pale yellowish solids in 74% yield, mp 272–275 °C (after LC); IR (KBr) ν_{max} = 3185, 2949, 1865, 1788, 1732, 1614, 1597, 1564, 1524, 1497, 1456, 1439, 1412, 1366, 1329, 1290, 1254, 1233, 1206, 1125, 1053, 1011, 991, 926, 854, 831, 810, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.47 (s, 1H), 9.25 (s, 1H), 9.20 (br s, 1H), 8.49 (d, *J* = 8.8 Hz, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 7.99 (d, *J* = 8.8 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 4.06 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 149.1, 148.3, 139.7, 135.6, 133.2, 126.3, 125.8, 122.9, 121.8, 120.6, 120.3, 120.1, 118.7, 115.4, 112.1, 52.9. HRMS calcd for $C_{17}H_{12}N_2O_2$: 276.0899, found: 276.0853.

Methyl 8-bromo-11*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (1b): Pale yellowish solids in 81% yield, mp 307–310 °C (after LC); IR (KBr) ν_{max} = 3163, 2953, 2214, 1881, 1794, 1724, 1614, 1593, 1564, 1522, 1479, 1445, 1408, 1366, 1333, 1292, 1273, 1258, 1229, 1206, 1115, 1044, 984, 926, 856, 831, 814, 764, 737 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 9.55 (d, *J* = 2.0 Hz, 1H), 9.31 (d, *J* = 2.4 Hz, 1H), 8.62 (d, *J* = 8.8 Hz, 1H), 8.48 (d, *J* = 1.6 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.56 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.99 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 148.8, 148.1, 137.9, 135.7, 132.8, 127.7, 126.0, 124.3, 122.7, 121.4, 120.0, 117.3, 114.9, 113.6, 112.0, 52.4. HRMS calcd for $C_{17}H_{11}BrN_2O_2$: 354.0004, found: 353.9977.

Methyl 6-methyl-11*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (1c): Pale yellowish solids in 85% yield, mp 245–248 °C (after LC); IR (KBr) ν_{max} = 3381, 3061, 2955, 1879, 1690, 1620, 1564, 1524, 1460, 1439, 1402, 1366, 1336, 1310, 1269, 1240, 1209, 1159, 1134, 1090, 1036, 988, 928, 856, 812, 772 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 9.49 (d, *J* = 1.2 Hz, 1H), 9.23 (d, *J* = 1.2 Hz, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.55 (s, 1H), 7.47 (t, *J* =7.6 Hz, 1H), 7.29 (t, *J* =7.6 Hz, 1H), 3.97 (s, 3H), 2.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 148.9, 148.4, 139.7, 139.5, 135.4, 132.7, 125.3, 123.2, 122.3, 120.9, 120.3, 120.1, 118.0, 113.7, 112.1, 52.7, 21.7. HRMS calcd for $C_{18}H_{14}N_2O_2$: 290.1055, found: 290.1040.

Methyl 6-phenyl-11*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (1d): Pale yellowish solids in 98% yield, mp 263–266 °C (after LC); IR (KBr) ν_{max} = 3377, 3057, 2953, 2361, 1723, 1694, 1620, 1559, 1528, 1495, 1454, 1362, 1323, 1310, 1275, 1238, 1198, 1123, 1103, 995, 934, 866, 812, 773, 745 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H), 9.66 (d, *J* = 2.0 Hz, 1H), 9.32 (d, *J* = 2.0 Hz, 1H), 7.70–7.59 (m, 7H), 7.41 (t, *J* = 7.6 Hz, 1H), 731 (d, *J* = 8.0 Hz, 1H), 7.04 (t, *J* =8.0 Hz, 1H), 4.00 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 148.9, 148.6, 142.6, 140.1, 140.0, 136.1, 133.1, 129.2, 129.1, 128.8, 125.6, 122.4, 121.7, 121.6, 120.4, 120.0, 116.6, 114.5, 112.3, 52.9. HRMS calcd for $C_{23}H_{16}N_2O_2$ $(M+H)^+$: 352.1212, found: 352.1248.

Oxidation of 10 with SeO_2 and rearrangement to form 11. To a solution of **10** (153 mg, 0.5 mmol) dissolved in MeCN:H_2O (3:1, 40 mL), heated to reflux, was added SeO_2 (277 mg, 2.5 mmol) portion by portion (five times every one hour) and the reaction was continued for 12 h, as monitored by TLC (hexane:THP = 2:1). After the reaction finished, MeCN was removed under vacuum. The residue was extracted with EtOAc (50 mL x 3), and the organic layer was collected and washed with saturated aqueous $NaHCO_3$ and brine to give **11** and **12** which were purified by flash column chromatography with the gradient of hexane: EtOAc = 10:1 to 1:1 v/v.

Methyl 5,6-dimethyl-11*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (11): Pale yellowish solids in 36% yield, mp 294–296 °C (after LC); IR (KBr) ν_{max} = 3362, 3051, 2953, 2922, 2854, 1871, 1703, 1609, 1566, 1508, 1449, 1418, 1373, 1350, 1327, 1302, 1265, 1229, 1134, 1109, 1086, 1049, 1028, 941, 873, 810 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H), 9.47 (d, *J* = 2.0 Hz, 1H), 9.28 (d, *J* = 2.0 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 7.2 Hz, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 3.98 (s, 3H), 2.90 (s, 3H), 2.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 147.5, 147.4, 139.8, 137.0, 133.7, 132.9, 125.1, 124.3, 123.3, 122.6, 120.4, 120.0, 118.1, 113.8, 112.0, 52.7, 18.2, 13.4. HRMS calcd for $C_{19}H_{16}N_2O_2$: 304.1212, found: 304.1263.

Methyl 6,6-dimethyl-5-oxo-6,11-dihydro-5*H*-pyrido[3,2-*a*]carbazole-2-carboxylate (12): yellow solid in 54% yield, mp 265–268 °C (after LC); IR (KBr) ν_{max} = 3364, 3281, 2959, 1732, 1711, 1688, 1603, 1551, 1439, 1408, 1366, 1325, 1302, 1261, 1248, 1134, 1092, 1005, 924, 878, 810, 750 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 9.04 (d, *J* = 2.0 Hz, 1H), 8.93 (d, *J* = 2.0 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 7.6 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 3.98 (s, 3H), 1.59 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 201.3, 165.1, 148.4, 146.1, 139.0, 131.2, 130.3, 129.2, 125.9,

125.4, 123.8, 120.7, 120.3, 120.2, 112.8, 53.3, 46.3, 26.9. HRMS calcd for $C_{19}H_{16}N_2O_3$: 320.1161, found: 320.1141.

Synthesis of 2-(methoxycarbonyl)-4-methyl-11*H*-pyrido[3,2-*a*]carbazol-4-ium iodide (13). A solution of **1a** (40 mg, 0.145 mmol) and MeI (82.2 mg, 0.58 mmol) in DMF (1 mL) was stirred at 60 °C for 9 h. Removal of the volatile under reduced pressure, the solids were washed with EtOAc and hexane several times to obtain the gray solids, 51 mg, 84% yield; mp >350 °C; IR (KBr) ν_{max} = 3451, 3078, 1721, 1632, 1601, 1547, 1503, 1458, 1435, 1393, 1368, 1333, 1294, 1273, 1252, 1221, 1165, 1126, 1105, 991, 937, 922, 797, 754 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 13.16 (s, 1H), 9.95 (s, 1H), 9.74(s, 1H), 8.95 (d, *J* = 8.8 Hz, 1H), 8.30 (d, *J* = 7.6 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.68 (d, *J* = 9.4 Hz, 1H), 7.56 (t, *J* = 7.2 Hz, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 4.68 (s, 3H), 4.10 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6, 147.5, 140.5, 139.5, 137.6, 135.1, 131.6, 122.2, 121.3, 121.1, 121.1, 120.9, 116.3, 112.4, 108.4, 53.4, 46.2. HRMS calcd for $C_{18}H_{15}IN_2O_2$: 418.0184, found: 418.0217.

Anti-proliferative activity screening test

Anti-proliferative assays in vitro were performed in the same manner as described in our previous papers.[32,33]

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Supporting Information

Experimental procedure and spectral data including ^1H NMR and ^{13}C NMR spectra of **8a**–**8d**, **10**, **1a**–**1d**, **11**, **12** and **13** are provided.

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